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A Rare Case of Triple-Negative Breast Cancer with RAD51D Gene Mutation

Annie Monica Ch[a], Rithwik Goud Burri[a], Sriharsha Lankala[a], Jun Yi Lim[b], Altynai Zhumabekova*[c]

[a]*Department of General Medicine, Maheshwara Medical College and Hospital, Hyderabad, Telangana, India*

[b]*Department of General Medicine, I. K. Akhunbaev Kyrgyz State Medical Academy, Bishkek, Kyrgyzstan*

[c] *AJ Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India*

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ABSTRACT

Background: The heterogeneity of breast cancer (BC) subtypes poses a significant challenge, with carcinogenesis involving multiple stages and genes, including proto-oncogenes, tumor suppressor genes, and DNA repair genes. Next-generation sequencing has expanded access to multigene panels, such as RAD51 paralogs, which increase the risk of ovarian cancer and possibly triple-negative (TN) BC.

Case presentation: We present a rare case of a 45-year-old woman with TNBC and a RAD51D gene mutation. Mammography and breast ultrasonography revealed an irregular 30 mm hypoechoic area and dystrophic calcifications in the right breast. Immunohistochemistry showed a lack of expression of ER, RP, HER-2, and P53, with 50% of neoplastic cell nuclei positive for Ki-67. Next-generation sequencing revealed a mutation in RAD51D and MUYTH genes. The patient underwent partial mastectomy, chemotherapy, and prophylactic mastectomy.

Conclusion: Genetic analysis is crucial for identifying specific mutations contributing to TNBC development. Current preventive interventions primarily address BRCA1 and BRCA2 mutations, following established guidelines.

INTRODUCTION

Breast cancer (BC) is a leading cause of death among women aged 40-59, accounting for an estimated 1.7 million new cases annually worldwide.[1] It represents 25% of all neoplasms and ranking as the second most common malignancy globally.[2] In addition to hereditary factors, female sex, and lifestyle choices, other risk factors affect the prevalence of BC.[3] A significant challenge in BC is the heterogeneity of many subtypes. The complex process of carcinogenesis involves multiple sequential stages and numerous genes, including proto-oncogenes, tumor suppressor genes, and genes associated with DNA repair.[4]

Next-generation sequencing (NGS) has expanded accessibility to multigene panels, such as RAD51 paralogs, which increase the likelihood of developing ovarian cancer (OC) and possibly triple-negative (TN) BC.[5] More than 40% of genetically determined BCs may be attributed to mutations in BRCA1 and BRCA2.[6] Therefore, identifying additional mutations is crucial. Genetic diversity presents a challenge in the development of targeted therapies. Mutations in BRCA1 and BRCA2 increase the risk of various cancers, and these genes interact with other genes to function as tumor suppressors in DNA transcription. Genetic mutations in BRCA1 and BRCA2 are

***Address for correspondence:**
Altynai Zhumabekova,
Honorary International Faculty, AJ Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India
Email:altynaizhm@gmail.com

responsible for 5–10% of all BCs and 20–25% of hereditary neoplasms.[7,8] These genes play a vital role in tumor suppression,[9,10] and when they malfunction, they disrupt the homologous recombination process, leading to genomic instability and an increased risk of developing various types of cancer, including BC, OC, and prostate cancers; pancreatic cancer; gastrointestinal tract tumors such as stomach, gallbladder, bile duct cancer; and melanoma.[11,12]

BC genes with moderate risk include CHEK2, ATM, PALB2, BRIP1, RAD51C, RAD51D, and BARD1. There are no established protocols for controlling the associated risk of additional malignancies. To provide screening advice, the patient's medical history and family background should be considered. We present a rare case of a patient with TNBC and RAD51D mutations.

CASE PRESENTATION

A 45-year-old woman without pre-existing health issues followed a balanced diet and had engaged in regular physical activity since the age of 15. She did not use a combination of oral contraceptives or intrauterine devices and had no history of pregnancy or miscarriage. Her grandmother had a family history of BC at the age of 60 years. The patient had a noticeable 2-3cm lump in her right breast during a doctor's visit. Breast ultrasonography and mammography revealed an irregular, 30mm hypoechoic area and dystrophic calcifications in the upper-outer quadrant of the right breast (Figure 1). These findings were classified as Breast Imaging Reporting and Data System 4. Additional tests confirmed invasive, grade 2 ductal carcinoma.

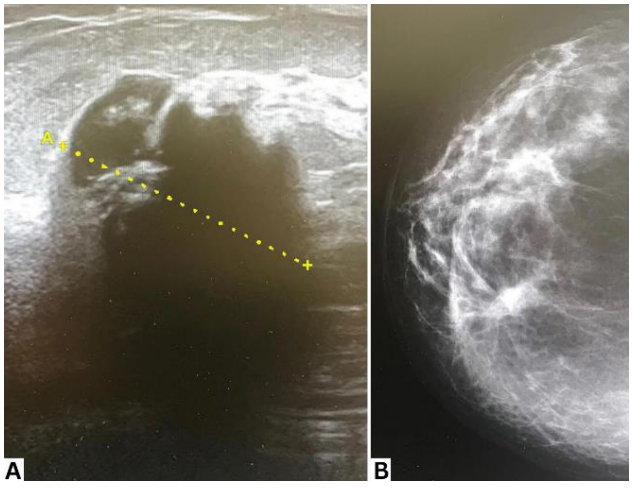

Figure 1. (A) Breast ultrasonography and (B) mammography revealed an irregular, 30 mm hypoechoic area and dystrophic calcifications in the upper-outer quadrant of the right breast

Core Needle Biopsy was performed using an image-guided core needle to extract breast tissue samples. Furthermore, formalin-fixed paraffin-embedded tissue sections were cut, deparaffinized, and rehydrated. Antigen retrieval was performed, followed by blocking of the nonspecific binding sites. These sections were incubated with primary antibodies, washed, and incubated with enzyme- or fluorescent dye-conjugated secondary antibodies. Enzyme-conjugated antibodies produced colored precipitates upon substrate addition, whereas the fluorescent antibodies were visualized microscopically. Counterstaining with dyes like hematoxylin provided contrast and morphological visualization. Coverslips were applied with mounting medium for preservation and examination. The invasive carcinoma in the excised specimen measured 2.5cm.

Immunohistochemistry showed a lack of expression of ER, RP, human epidermal growth factor receptor-2 (HER2), and P53 [mutant (null) pattern], but 50% of neoplastic cell nuclei were positive for Ki-67. Partial mastectomy was performed in the upper quadrant of the right breast along with excision of the three sentinel axillary lymph nodes. The patient's staging was validated using chest, abdomen, pelvic tomography, and bone scintigraphy, which revealed a pT2pN0M0 classification, stage II-A. A clinical geneticist was consulted, but molecular alterations in BRCA1 and BRCA2 were not observed.

The patient was sent for further examination, which revealed a mutation in RAD51D and MUYTH. Therefore, NGS was recommended. PacBio (Pacific Biosciences, California, United States), an NGS platform, was used to study the RAD51D mutations. This process involved sample preparation, library construction, sequencing, and data analysis. DNA was extracted from the tissue samples, and its quality and quantity were evaluated using spectrophotometry and gel electrophoresis. DNA libraries were prepared using the Illumina TruSeq DNA Library Prep Kit, and included fragmentation, end repair, A-tailing, adapter ligation, and PCR amplification. The libraries were sequenced on an Illumina platform with 150 bp paired-end reads. Raw sequencing data quality was checked using FastQC and high-quality reads were aligned to the human reference genome (GRCh37/hg19) using BWA-MEM. Variants were called using GATK HaplotypeCaller and annotated using ANNOVAR. The sequencing output included FASTQ files with raw reads, BAM files aligned to the reference genome, and VCF files with identified variants. These formats allow for a comprehensive analysis and verification by other researchers. Supplementary materials or appendices accompanying research articles provide detailed methods or specific NGS data queries, and can illustrate typical findings if specific raw data

segments are required. The sequencing data were compared with the GRCh37/hg19 version of the human genome and showed a substitution of the amino acid Arginine with Glutamine at position 232 in RAD51D and a substitution of the amino acid Arginine with Histidine at position 217 in MUYTH. However, the MUYTH mutation is "variant with unknown or uncertain significance". The genes and their detection methods are presented in Table 1.

Table 1. Genes mentioned and their detection methods in the case

Gene	Full name	Detection Method
ER	Estrogen Receptor	Immunohistochemistry (IHC)
PR	Progesterone Receptor	IHC
HER-2	Human Epidermal Growth Factor Receptor-2	IHC
P53	Tumor Protein P53	IHC
Ki-67	Marker of Proliferation Ki-67	IHC
BRCA1	Breast Cancer 1	Next-Generation Sequencing (NGS)
BRCA2	Breast Cancer 2	NGS
RAD51D	RAD51 Paralog D	NGS
MUYTH	MUTYH Glycosylase	NGS

IHC: Immunohistochemistry; NGS: Next-Generation Sequencing.

Chemotherapy consisted of an adjuvant regimen of doxorubicin and cyclophosphamide followed by paclitaxel treatment. The patient was admitted to the hospital during the second cycle due to febrile neutropenia, which was treated with antibiotic prophylaxis. The patient was discharged after one week, and the remaining chemotherapy cycles were completed without complications. After receiving genetic counseling, the patient underwent prophylactic mastectomy of the right breast and awaited the same procedure for the left breast. The patient's ovaries were removed at the age of 40 as a preventive measure. Since the patient is childless, she decided to cryopreserve the egg.

DISCUSSION

TNBC is a rare and aggressive type of BC that does not contain hormone receptors for the estrogen, progesterone, or HER2 protein. As a result, this type of BC does not respond to typical hormone treatments or targeted medications and tends to spread to internal organs with a higher likelihood of brain metastasis. It also has a higher recurrence rate and is often diagnosed at a young age.[13] Genetic analysis is crucial for identifying the specific genetic mutations that contribute to its development, as it has a higher recurrence rate and is often diagnosed at a young age. TNBC commonly has strong and rare hereditary mutations in BRCA1 and BRCA2, which significantly increase the risk of BC. These tumors have characteristics similar to those of typical TNBC tumors in terms of their shape, recurrence patterns, and death rates.[14,15] Studies of TN patients have also shown that approximately 20% of cases diagnosed before the age of 50 years have inherited BRCA1 mutations.[16] BRCA1 mutations lead to DNA errors, genomic instability, and increased risk of cancer. Cluster microarray testing of RNA expression data showed high correlation between TNBCs and BRCA1 cancers, indicating the presence of similar cancer pathways. TNBCs have unique molecular profiles and limited responsiveness to molecular therapy.[17]

Patients with the RAD51D germline mutation have an estimated 20-23% lifetime risk of breast cancer by the age of 80 years, particularly for TNBC, which lacks estrogen and progesterone receptors and HER2 expression. This complicates treatment and often requires aggressive approaches, highlighting the importance of early detection and preventive strategies for individuals with this genetic alteration.[18-20]

It has been reported that RAD51C and RAD51D are involved in the FA-BRACA1/2 pathway.[5] The Fanconi anemia-BRCA (FA-BRCA) pathway, involving 16 FA proteins and BRCA1/2, is crucial for repairing DNA interstrand crosslinks (ICLs) and maintaining genomic stability.[21,22] The FA core complex, comprising eight FA proteins, facilitates monoubiquitination of FANCD2 and FANCI, promoting their recruitment to DNA damage sites and coordinating ICL repair. Pathway dysfunction leads to cancer susceptibility in Fanconi anemia or BRCA1/2 mutation carriers.[21,23] The FA-BRCA pathway is a complex network that protects cells from genotoxic stress by detecting, signaling, and repairing ICLs. Its disruption increases sensitivity to DNA-damaging agents, whereas reactivation is associated with acquired drug resistance, highlighting its importance in cancer biology and therapy.[22]

NGS and oncogenic research have enhanced the implementation of preventive interventions for cancer-causing mutations and predisposition syndromes. Current methods primarily address

mutations in BRCA1 and BRCA2 following the National Comprehensive Cancer Network guidelines. Women with these mutations should undergo clinical breast examinations every 6 to 12 months, annual magnetic resonance imaging of the breast starting at age 25, annual mammography starting at age 30, and annual transvaginal ultrasound and serum CA-125 concentration tests.[24]

Bilateral risk-reducing mastectomy and bilateral risk-reducing salpingo-oophorectomy suitability assessments are typically conducted between 35 and 40 years of age after pregnancy. Patients with hereditary BRCA1 and BRCA2 mutations may benefit from Poly (ADP-Ribose) Polymerase (PARP) inhibitor therapy, which exploits the synthetic lethality. Additionally, somatic BRCA1 and BRCA2 mutations are found in various non-hereditary cancers, and these tumors can also be effectively treated with PARP inhibitors because their molecular features are similar to those of hereditary cancers.[25]

There is a lack of definitive evidence regarding bilateral mastectomy indications for patients with RAD51D gene mutations. RAD51D, which is crucial for DNA repair, is linked to a higher risk of ovarian cancer; however, its association with breast cancer risk and surgical decisions remains unclear.[26] In contrast, bilateral mastectomy for BRCA1/2 mutation carriers, which are known breast cancer risk factors, is better documented. For these patients, bilateral mastectomy is a risk-reducing option, although it does not improve survival compared with breast-conserving therapy or unilateral mastectomy.[27,28] Younger age, family history, and BRCA mutations influence the decision to undergo bilateral mastectomy.[29-31] Thus, while bilateral mastectomy is recognized for high-risk BRCA mutation patients, guidelines for RAD51D mutation carriers have not yet been established. Consequently, decisions for these patients rely on broader genetic risk assessments and individual factors rather than the specific RAD51D literature.

A study by Torres-Esquius *et al.* highlights the high prevalence of estrogen receptor-negative phenotypes among breast cancer cases with RAD51C and RAD51D mutations, which are particularly aggressive and currently lack targeted therapies beyond PARP inhibitors.[32] Approximately 15% of patients with OC have GPVs in BRCA1, BRCA2, MLH1, MSH2, MSH6, BRIP1, PALB2, RAD51D, and RAD51C. Guidelines exist for managing cancer risk in patients with GPVs in BRCA1, BRCA2, MLH1, MSH2, and MSH6 but not for BRIP1, PALB2, RAD51D, and RAD51C, leading to uncertainty regarding the timing and appropriateness of risk-reducing surgeries. Recent exploration of the link between RAD51C, RAD51D, and breast cancer remains unclear.[33]

RAD51D mutation is a significant factor in the development of OC and TNBC, making it relevant to the patient in question. Studies have estimated the cumulative risk of OC to be about 1% at the age of 40 and rising to 14% by the age of 80.[5] However, before the current investigation, there was a lack of scientific data supporting the heightened risk of BC in women with RAD51D mutations.[34]

It is crucial to raise awareness of this information among the female relatives of affected individuals, as it allows them to make informed choices about preventive measures and early screening alternatives. In the case of the patient in question, an interdisciplinary approach to monitoring facilitated decision-making regarding preventive intervention. She opted for egg freezing and oophorectomy, which were supported by the diagnosis made by gynecology, therapy recommended by oncology, and counseling provided by medical genetics. These interventions ultimately lead to positive outcomes in the clinical setting.

CONCLUSION

This case examined the association between moderately prevalent mutations, particularly those involving RAD51D and BC. Therefore, it is important to develop accurate preventive and treatment methods for patients carrying this genetic mutation. NGS provides diverse applications and is consistent with the importance of genetics and multidisciplinary monitoring of patients with BC. We suggest further exploration in the field to facilitate the sharing of scientific evidence on epidemiological connections.

ETHICAL CONSIDERATIONS

The patient was fully informed about the details of her disease being presented in this journal, and she signed an informed consent form.

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CONFLICT OF INTEREST

No conflicts of interest exist regarding the publication of the present study.

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